Global Microbial Threats

Emerging Infections
Ebola and Other Filoviruses

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The images of the bizarre forms of Ebola virus have become familiar to many of us (Figure 1), but the effect of these unique forms on the discoverers of the first known filovirus, the Marburg virus, must have been startling. When the Marburg virus was isolated in 1967, these elongated, irregularly shaped virus particles had the additional context of a new disease with a high mortality transmitted to those caring for the original patients. All that was known was that the virus was imported from Uganda with wild-caught African green monkeys and that it represented a previously unknown group of viruses. What have we learned in the intervening years, and how should we regard these agents?

We now know that the Marburg and Ebola viruses are members of a new virus family, Filoviridae. This family was defined through the morphologic and replicative strategies of the viruses, as contrasted to other single, negative-strand RNA viruses. Their natural history has remained unknown and their clinical appearances infrequent. Indeed, filoviruses are known only from a few isolates in Africa over the intervening years, including those of the Ebola virus in Zaire (1976, 1979, 1995),[1,2] Sudan (1976, 1979), and Ivory Coast (1994) and the Marburg virus in Zimbabwe (1975) and Kenya (1980, 1984, 1987). A series of isolates of another Ebola subtype was identified from 1989 to 1992 from monkeys imported from the Philippines into Reston, Virginia, and other sites in the United States and Siena, Italy.[3] The infrequent isolations are more remarkable for the fact that the Ebola "virus" is actually composed of four subtypes that are 30% to 45% different at the nucleotide level, suggesting that they represent four different viruses. Thus, each filovirus episode, including the three large epidemics (Sudan and Zaire in 1976 and Zaire in 1995),[1,2] is thought to represent only a single or limited introduction of the Ebola virus into the human population with subsequent interhuman transmission. This is likely also the case for the Reston, Virginia, monkey episode in 1989.

Arenaviruses provide an interesting comparison. The identification of Junin and Machupo viruses from cases of Argentine and Bolivian hemorrhagic fever also led to the definition of a new virus family Arenaviridae. The basis for including these viruses with lymphocytic choriomeningitis and Tacaribe viruses was primarily morphologic and serologic. After the definition of the family, new members such as the Lassa fever virus were quickly added. Today there are at least 14 arenaviruses discovered from the Americas, and at least 5 are human pathogens. They all cause chronic infections of rodents, with the possible exception of Tacaribe virus, which is known only through isolates from bats on the island of Trinidad. Arenaviruses can be found regularly in many cases by revisiting previous sites of activity, and cases of both Argentine hemorrhagic fever and Lassa fever appear yearly in the pampas and in west Africa, respectively. Thus, arenaviruses have followed the usual historical course associated with the identification of a new group of viruses; their reservoirs are known, and we understand at least the basics of their transmission in nature. For Argentine hemorrhagic fever and Lassa fever, we have observed thousands of cases of infection in humans from the natural reservoir and the consequences, both clinically and in terms of interhuman transmission. This provides us with a starting point in developing the principles to control human disease and contrasts markedly with the filovirus situation.

Another contrast between arenaviruses and filoviruses lies in the area of treatment. Arenaviruses are sensitive to the antiviral drug ribavirin in cell culture; Lassa fever, and probably other arenavirus diseases, can be successfully treated with the drug. Convalescent plasma is highly effective in therapy for Argentine hemorrhagic fever. Filoviruses have not been susceptible to inhibition in vivo or in vitro by ribavirin. Furthermore, their in vitro neutralization by convalescent plasma is negligible or inefficient, and attempts to treat animals with convalescent plasma have uniformly failed. There is a single rather unconvincing case report of the administration of interferon and convalescent plasma to a person infected with the Ebola virus in the laboratory, and studies of nonhuman primates suggest that hyperimmune horse serum might be effective in the postexposure prophylaxis of Ebola virus infection of baboons.

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ABBREVIATIONS USED IN TEXT

ELISA = enzyme-linked immunosorbent assay
Ig = immunoglobulin

In May 1995, the world was faced with another Ebola virus outbreak, this time in the large urban center of Kikwit, Zaire (Figure 1).[1] Several valuable diagnostic modalities developed as a result of the 1989 Reston, Virginia, importation of Ebola-infected monkeys served effectively. The results of antigen-capture enzyme-linked immunosorbent assay (ELISA) were positive in 11 of 13 acutely infected patients from whom specimens were initially taken, and this provided a timely and accurate measure of infection. Immunoglobulin (Ig) M-capture ELISA detects antibodies in recently convalescent patients, and IgG-ELISA seems to have overcome the problems of nonspecificity that have plagued the indirect fluorescent antibody test. The final evaluation of these tests awaits the lengthy process of analyzing specimens by conventional virologic methods. This will also provide the first description of the clinical process of Ebola viral infection in humans; unfortunately, the number of adequate serial specimens collected is limited. These tests are robust enough and free of problems of cross-contamination that they could be taken to the field if trained staff were available and the need were sufficient.

Reverse transcription and polymerase-chain reaction amplification of viral RNA from serum proved to be highly sensitive and had the added advantage of allowing the direct sequencing of the genomic fragment and a definitive diagnosis of the presence of the Zaire subtype of the Ebola virus.[1]

The finding by immunohistochemical staining of extensive skin involvement in human specimens from this epidemic provides both a new diagnostic modality and interesting insights into a possible epidemiologic role for contact contamination (Sherif Zaki, unpublished observations). Moving acute viral specimens from remote African sites to reference laboratories is difficult. Dry ice or liquid nitrogen are usually not available, and the strict International Airline Transport Association rules implemented in January 1995 are complicated and require packing materials that are expensive and not generally available. Thus, the simple step of being able to fix a skin biopsy specimen in formalin and transport it without these considerations opens the way to a more feasible surveillance mechanism, particularly in a disease with a mortality that exceeds 75%, making antibody studies on survivors difficult.

The control of the epidemic required the collaboration of many organizations and strained the budgets and resources available. Thanks to the dedication and efficiency of many courageous Zairians and international workers, it was possible to contain the disease, but not without at least 318 cases, a mortality of 77%, and abortive exportation of the virus to nearby villages. One person infected in Kikwit reached the capital Kinshasa, but there was no further transmission. Dealing with this infectious disease in a town of 250,000 with poor communications and little medical infrastructure was a challenge that fortunately was met successfully. Formal epidemiologic studies of interhuman transmission, still under analysis, confirmed and extended the impressions from previous epidemics.[1,2]

The first reliably identified case occurred in a charcoal worker from Kikwit who died in January 1995, four months before the epidemic was diagnosed. Viral transmission between humans involved close contact with a patient, and proper barrier nursing protected the medical staff. As an example of how difficult it can be to ensure the termination of transmission in such an urban setting,

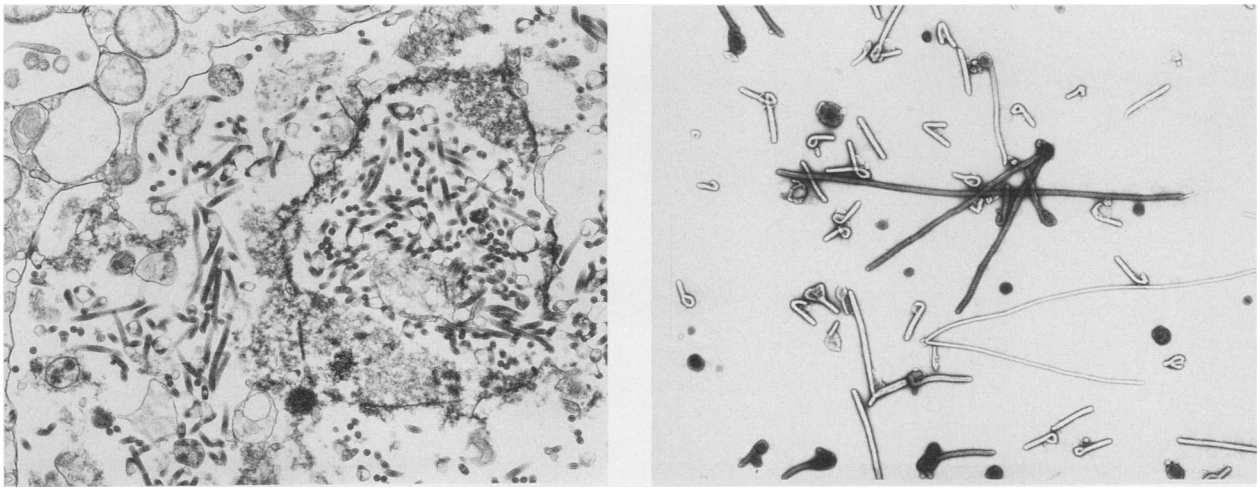

Figure 1.—Electron micrographs of the 1995 Zaire isolate of Ebola virus: **Left,** A thin section of infected Vero cell shows massive production of virions in longitudinal and cross-section. **Right,** A negative stain of whole virions shows shorter replicative forms and longer, irregularly shaped structures. (Courtesy of Charles Humphrey and Cynthia Goldsmith, Cellular and Molecular Pathology Activity, Division of Viral and Rickettsial Diseases, Centers for Disease Control and Prevention.)

immunohistochemical staining of skin specimens revealed one case a month after all participants had agreed that transmission had ceased based on the usual surveillance mechanisms.

Unfortunately, ecologic studies, which were carried out in a collaborative effort among scientists from the United States, Zaire, Belgium, South Africa, and other countries, have not yet yielded any information as to the true virus reservoir that infected the index case of the epidemic. Analysis of the material collected in Zaire will require a major effort and many months of work with no guarantee that any useful positive information will emerge. All laboratory work must be done under biosafety level 4 or maximum-containment conditions in special laboratories to prevent the virus from escaping.

Filoviruses continue to provide a difficult area for virologists to develop strategies to protect the public and can be seen as the prototype of emerging viruses. We do not understand their natural maintenance strategy and thus cannot predict their emergence nor the factors that might reasonably be expected to increase the risk of their presenting problems to the world. Given our profound ignorance of these viruses, the limited number of episodes we have studied, and their lethal potential, it seems a safe bet that we have additional unpleasant surprises in store. The task now is to garner continuing support to understand these elusive agents now that the epidemic has been controlled and public interest has faded.

I would like to acknowledge the many participants in the control and study of the epidemic in Kikwit, some of whom are still working to finish testing of specimens in the laboratory, analyzing data, and preparing detailed and definitive accounts for publication.

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